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Lipoprotein-associated PLA_2 inhibition — a novel, non-lipid lowering strategy for atherosclerosis therapy

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Abstract

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a serine lipase that is associated with low density lipoprotein (LDL) in human plasma. Substrates include oxidised phosphatidylcholine (PC), which is hydrolysed by Lp-PLA_2 to lyso-PC and oxidised fatty acids. Both products are bioactive and proinflammatory, and implicated in monocyte infiltration into the developing plaque, deposition of foam cells, and plaque progression and instability. Lp-PLA_2 has recently been shown to be a risk factor for coronary events in previously asymptomatic, hypercholesterolaemic men. A series of azetidinones was designed as potent and selective inhibitors of this enzyme; SB-222657 inhibited release of the chemotactic cleavage products from oxidised LDL, and SB-244323 reduced atherosclerotic plaque development in a 3 month rabbit study. A series of pyrimidones has been designed from a screening hit, and nanomolar inhibitors identified. Oral efficacy in inhibiting plasma Lp-PLA_2 in rabbits has been demonstrated with a variety of structural classes.

Keywords: Phospholipase A_2; Lp-PLA_2; Atherosclerosis therapy; Enzyme inhibitors

1. Disease target

Cardiovascular disease (CVD) is a major cause of death in western populations, and atherosclerosis is the fundamental pathology behind most CVD deaths. Interest in potential therapies for this disease is correspondingly great, but until recently most strategies have aimed to address the dyslipidaemias frequently observed in high-risk populations, by reducing plasma cholesterol and triglycerides. HMG-CoA reductase inhibitors ('statins') have proved effective in this role, and have come to dominate the market for anti-atherosclerotic drugs. However, trials have shown that statins are effective at preventing outcomes such as myocardial infarction (MI) in only 30–40% of the patient group [1], and that many MI patients had a normal lipid profile. There thus appears to be a need for new therapies with actions synergistic to lipid-lowering agents such as the statins.

As the pathogenesis of atherosclerosis has become somewhat better understood, attention has started to shift to processes occurring within the plaque as possible targets for drug intervention. In particular, it has become apparent that atherosclerosis can be regarded as a localised inflammation of the artery wall, and plaque progression is substantially driven by inflammatory mediators [2,3]. Although early stage plaque tends to be asymptomatic, advanced plaque resulting from chronic inflammation becomes unstable and gives rise to adverse clinical events. Plaque stabilisation is thus a key target for future therapies.

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a calcium-independent serine lipase that, in plasma, is mostly associated with low density lipoprotein (LDL) particles. Its substrates include platelet-aggregating factor (PAF), and the enzyme is widely referred to in the literature as PAF acetylhydrolase, but its substrate specificity is much broader than this name implies [4,5]. Oxidised LDL (formed under conditions of oxidative stress, such as exist in the artery wall [6]) contains a variety of lipid peroxidation products, which are efficiently hydrolysed at the SN2 position by Lp-PLA_2. The products are oxidised fatty acids plus lyso-phosphatidylcholine (PC) (Fig. 1); both have a variety of proinflammatory actions, including monocyte chemoattraction.

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A proposed proatherogenic mechanism for this enzyme is shown in Fig. 2. LDL, with associated Lp-PLA_2, becomes oxidised within the intima, providing a substrate for the enzyme. The resulting hydrolysis products promote the chronic inflammation that is associated with macrophage accumulation. Macrophages also produce and secrete further Lp-PLA_2, representing one of the positive feedback mechanisms driving disease progression.

Lp-PLA_2 also has potential antiatherogenic actions via clearance of PAF [7], but support for the view that proatherogenic mechanisms dominate came from the West of Scotland Coronary Prevention Study (WOSCOPS). In a cohort of 580 cases with CHD, matched with 1160 controls, it was shown that Lp-PLA_2 was not correlated with other inflammatory markers (CRP, fibrinogen, white cell count), and there was a strong, positive correlation between Lp-PLA_2 level and coronary events [8]. The study group concluded that chronic inflammation is a strong determinant of risk in asymptomatic, hypercholesterolaemic men, and that Lp-PLA_2 is a new, independent marker of CHD risk.

2. Acylating inhibitors

Little is known about the three-dimensional structure of Lp-PLA_2 that could guide rational design of inhibitors, but the enzyme appears to be a serine hydrolase with a catalytic triad analogous to many proteases.

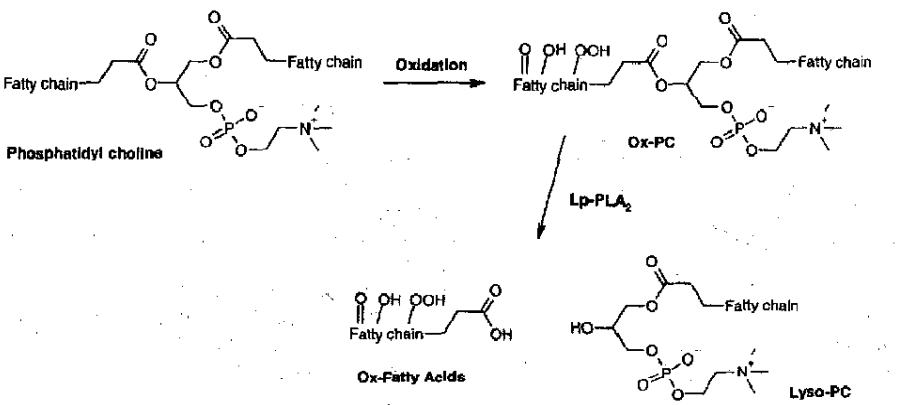


Fig. 1. Oxidation and hydrolysis of PC.

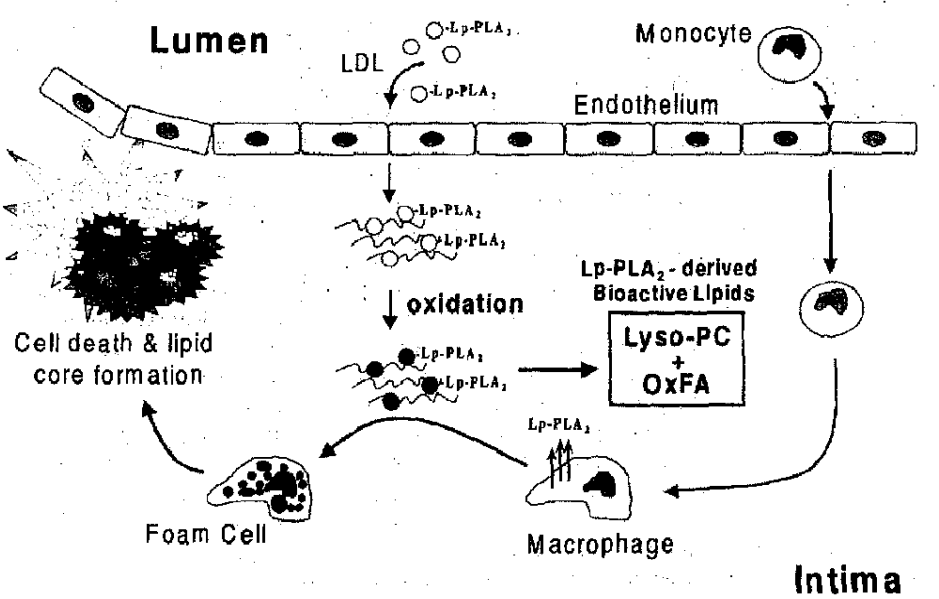
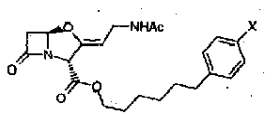


Fig. 2. Postulated role of Lp-PLA_2 in atherogenesis.

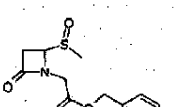
We therefore cross-screened a variety of serine protease inhibitors against the lipase, and identified clavulanate esters as a promising lead. After some SAR optimisation these led to highly potent inhibitors (**1**), which act by acylation of the active site serine, but chemical instability limited the value of this series for biological studies. Attention shifted to the more stable azetidinone series, starting from the weakly active lead **2**. Changing the ester to a more stable amide, optimising the chain length of the N-substituent and modifying the sulfur substituent improved potency 1000-fold, and SB-222657 (**3**) was selected for further biological studies.

The oxidative processes affecting LDL within the artery wall can be simulated in vitro by Cu^{2+}-catalysed oxygenation, producing a good substrate for the Lp-PLA_2 present. Such oxidation of the LDL fraction from human plasma thus gives lyso-PC as one of the products. SB-222657 inhibits this lyso-PC production in a dose-dependent manner, with an IC_{50} of 11.7 nM [9]. The free fatty acid fraction from oxidised LDL has markedly greater monocyte chemotactic activity than unoxidised controls, and it was shown that SB-222657 inhibits this activity to baseline levels, with an IC_{50} of 5.0 nM. Lp-PLA_2 activity in the same incubations was inhibited with an identical IC_{50} of 4.9 nM [9].

These in vitro studies confirm that SB-222657 affects oxidised LDL as predicted from the mechanism in Figs. 1 and 2. However, the poor pharmacokinetic profile of this compound made it unsuitable for in vivo dosing. Further optimisation led us to SB-245713 (**4**), with a relatively low in vivo clearance rate and (as the sodium salt) good solubility in intravenous (iv) formulations. The acid and sodium salt still had poor oral bioavailability, but it was found that the ethyl ester (**5**, SB-244323) was an effective prodrug: after oral administration to rabbits, little of the ester was seen in plasma, but there were high circulating concentrations of acid **4** (Schemes 1 and 2).

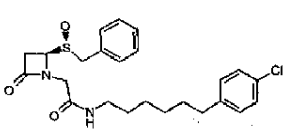


1, IC_{50} 2-10 nM

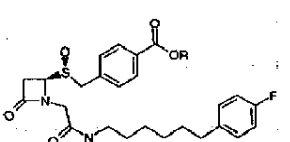


2, IC_{50} 3000 nM

Scheme 1.



3 (SB-222657), IC_{50} 3.7 nM



4, R = H, SB-245713, IC_{50} 5.2 nM
5, R = Et, SB-244323

Scheme 2.

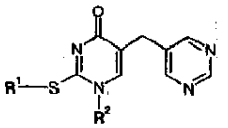
Initial studies with WHHL rabbits showed that SB-244323 could be dosed in the diet, producing almost complete inhibition of plasma Lp-PLA_2 activity. More significantly, PLA_2 activity in washed and homogenised aortic segments was extensively inhibited relative to control animals, giving us some confidence that the compound was able to diffuse into the artery wall and act at the site of the developing plaque. On this basis, we proceeded with a 3 month proof of concept study in WHHL rabbits [10]. 20 μmol/(kg day) diet dosing of SB-244323 produced > 99% inhibition of plasma PLA_2 and 54% inhibition of aortic PLA_2. Despite this virtual knockout of the circulating enzyme, all the rabbits survived the dosing period, and no adverse effects were observed. Histological analysis of aortic segments showed a decrease in both lesion cross-sectional area and thickness, particularly in segments with the most complex, raised plaque. This perhaps suggests that Lp-PLA_2 inhibitors are most effective in blocking the later stages of plaque progression, including stability.

3. Non-covalent inhibitors

High-throughput screening of the SB compound collection against Lp-PLA_2 identified a series of pyrimidones, which, in contrast to the azetidinones, were freely reversible, substrate-competitive inhibitors in kinetics experiments. After initial optimisation as described elsewhere [11], we obtained two lead structures **6** and **7**. However, when these compounds were tested against endogenous Lp-PLA_2 in human and WHHL rabbit plasma [12], they proved to be much less effective inhibitors than when using purified enzyme in buffer (Table 1). This is presumed to be because of non-specific binding to plasma proteins. Significant activity in the plasma assays was only achieved after we investigated N-substitution of the pyrimidone ring. The 3-methyl derivatives were essentially inactive, but the 1-methyl isomers (**8** and **9**) were somewhat more potent than the NH compounds **6** and **7** against isolated enzyme, and substantially better in plasma. Notably, **9** was at least as effective in plasma as the long-chain compound **8**, despite a 30-fold potency drop in the enzyme assay, and most further optimisation was based on this benzylthio series.

Perhaps unsurprisingly for a lipase, enzyme affinity could be increased significantly by adding long hydrocarbon chains (Table 2). However, potency in plasma was only improved by introducing polar groups within the chain, as in acetamides **14** and **15** or butyramide **16**. Attempts to mimic natural lipids with long, unsaturated chains gave no further improvement in vitro, but **17** and **18** were particularly impressive on oral dosing to WHHL rabbits [12].

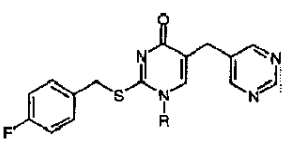
Table 1
In vitro Lp-PLA_2 activity of lead structures



	R^1	R^2	IC_{50} (μM)[a]	Inhibition in plasma (%)[b]	
				Human 0.3 μM	Rabbit 1 μM
6	4-Cl-$C_6H_4CO(CH_2)_7$	H	0.054	2	0
7	$PhCH_2$	H	1.1	4	4
8	4-Cl-$C_6H_4CO(CH_2)_7$	CH_3	0.019	53	23
9	$PhCH_2$	CH_3	0.66	54	33

[a] Using purified Lp-PLA_2 [11].
[b] Endogenous PLA_2 activity in plasma from WHHL rabbits or human volunteers [12].

Table 2
Effect of varying N1-substitution on in vitro Lp-PLA_2 activity



	R	IC_{50} (nM)	Inhibition (%) in plasma at 100 nM	
			Human	Rabbit
10	H	850		
11	Me	280	28	6
12	$(CH_2)_9Ph$	25	10	10
13	*n*-Undecyl	19	11	10
14	$CH_2CONHC_8H_{17}$	7	43	68
15	$CH_2CONHC_{12}H_{25}$	0.3	86	54
16	$(CH_2)_3CONHC_{10}H_{21}$	0.1	94	77
17	(*E*) $(CH_2)_3CONH(CH_2)_6CH{=}CHC_8H_{17}$	0.7	71	47
18	(*Z*) $(CH_2)_3CONH(CH_2)_8CH{=}CHC_8H_{17}$	0.4	85	59

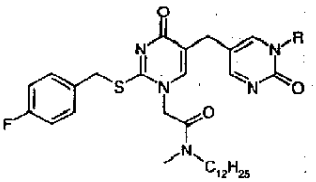
Such lipid mimics inevitably had poor aqueous solubility, so we also attempted to prepare less-lipophilic inhibitors. The pyrimidine could be replaced with much more polar groups, such as the 2-oxo derivatives in Table 3. In particular, the sodium salt of acid **22** proved highly water-soluble (> 25 mg/ml in saline at pH 7.4), and was well suited to studies requiring an iv formulation.

Even these compounds have relatively high calculated log *P* (see Ref. [13]) values, so we also searched for alternatives to the long-chain *N*-alkyl amide group. Significant lipophilic character in this part of the molecule appears to be a requirement for high potency, but the piperazines in Table 4 show one way to strike a balance in properties. The piperazine acetamide group of **23** contributes little enzyme affinity (cf. **10**, calculated log *P* 1.1), but *N*-aryl derivatives such as **25** and **26** are substantially more potent while retaining calculated log *P* values in the 'drug-like' range. Interestingly, adding further lipophilic groups gave, at best, mixed results; e.g. the dichloro derivative **27** had a lower IC_{50} than **26**, but was less good in plasma. The monochloro compound was also more effective in WHHL rabbits: peak inhibition seen after a 10 mg/kg oral dose was 57% for **26** versus 37% for **27**.

4. Conclusions

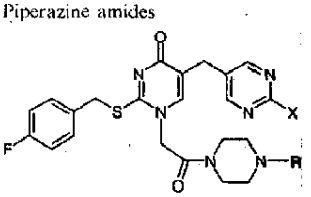
Lp-PLA_2 appears to play a key role in atherogenesis, which merits more intensive study. We have identified potent, selective inhibitors in two distinct classes and with a variety of physicochemical and enzyme mechanistic properties. As tool compounds, these are allowing

Table 3
2-Oxopyrimidines



	R	calculated log *P*	IC_{50} (nM)	Inhibition (%) in plasma at 100 nM	
				Human	Rabbit
19	H	5.3	0.9	83	39
20	CH_3	5.7	5.0	75	32
21	CH_2CH_2OH	5.4	1.0	77	36
22	CH_2COOH	5.9	1.0	74	28

Table 4
Piperazine amides



	R	X	calculated log *P*	IC_{50} (nM)	Inhibition (%) in plasma at 100 nM	
					Human	Rabbit
23	H	H	0.8	460	nd	nd
24	Ph	H	1.8	120	38	22
25	4-Cl-Ph	H	2.7	26	73	37
26	4-Cl-Ph	MeO	3.2	20	77	33
27	3,4-di-Cl-Ph	MeO	3.9	8	67	22

us to investigate the pharmacology of the developing plaque in novel ways. Work is continuing, in this exciting area, to identify drugs suitable for clinical use.

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